Biochemical investigation and anticonvulsants activity of green synthesized Nanoparticles using *in vivo* plantlets and *in vitro* grown tissues of *Withania somnifera* (L.) Dunal and *Bacopa monnirei* (L.)Wettst.

For the Degree of Doctor of Science in Botany

Submitted by

Dr. Suchitra Mahato
Assistant Professor and Head
Department of Botany
Silli College, Silli
(Under Ranchi University, Ranchi)

Under the Supervision of
Dr. (Mrs.) Anita Mehta
Associate Professor
University Department of Botany
Ranchi University, Ranchi

State of Art-

Since ancient times, plants have been used by mankind for their healing and therapeutic abilities, and we still depend on their medicinal properties today. It is well known that medicinal plants play a key role in the development and advancement of modern drugs[1,2]. Approximately 25% of drugs used in modern Pharmacopoeia are derived from plants and many others are synthetic analogues built on prototype compounds isolated from plants. Many plants contain active compounds that have direct pharmacological effects on our bodies, including various organs. One such major organ is brain, so complex that still only few drugs are approved by drug authorities for ailments like epilepsy. Epilepsy is chronic, non-communicable neuropsychological disorder of brain caused by an excessive release of neurotransmitters. It is characterized by recurrent, unprovoked seizures. These seizures are caused by abnormal electrical activity in the brain, leading to a wide range of symptoms depending on which parts of the brain are involved. Symptoms can include temporary confusion, staring spells, uncontrollable jerking movements of the arms and legs, loss of consciousness or awareness, and cognitive or emotional disturbances.

Epilepsy can develop at any age and most cases of epilepsy are idiopathic, although in some cases they result from brain injury, stroke, brain tumor, genetic mutations, developmental disorders, severe malaria and drug abuse. [3]

The severity of epilepsy and the frequency of seizures can vary widely between individuals. A link between epilepsy and cognitive deterioration has also been observed. Ogunarin [4] noted that defective memory, especially for recent acquisitions and weakened capacity for attention are frequently encountered cognitive impairments in epileptic patients, Epilepsy is one of the most common and serious disorders of the brain [5]. About 1% of the population suffers from epilepsy, and about one-third of patients have refractory epilepsy (i.e., seizures are not controlled by appropriate antiepileptic medications). Epilepsy can result in significant injury as well as death, if untreated.

It is a major public health issue especially in developing countries. The WHO estimates that there are 50 million people living with epilepsy (PWE) worldwide, making it a universal global neurological problem [6] . Eighty percent of PWE are distributed in low- and middle-income countries. Consequently, it is estimated that 10–12 million PWE are living in India, contributing to almost one-sixth of disease load. The risk of having epilepsy at some point in the average life span of any individual varies between 2% and 5%. Epilepsy is classified as *Apasmara* by Ayurvedic system of medicine, apa, meaning negation or loss of; smara, meaning recollection or consciousness.

Drugs commonly known as anticonvulsants have been used for the treatment of epilepsy, which has shown up to unpredicted and undesired side effects, chronic toxicity, and defects in giving birth due to the long-time therapy exposed to patients with epileptic conditions and still patients remain unwell [7,8,9].

Using herbal drugs as an additional treatment for epilepsy is gaining popularity because they tend to have fewer side effects. These herbal drugs work at the target site with the same mechanism of action as synthetic drugs. For the management of psychiatric conditions including migrane, epilepsy, alzhemer's anxiety, parkinson's depression and so on, ayurveda goes on to describe the use of selected species and plant based preparation[10.11.12] .

Many plants used in traditional medicine for epilepsy are now being scientifically studied to confirm their anticonvulsant activity [13,].

While natural remedies are efficacious and benign in nature and are among the most popular ones of complementary and alternative medicine. The use in the treatment of neuro brain disorders is already receiving attention. Medicinal herbs preparation exhibit its poly-pharmacological influence using its multi-component matrix to operate on multiple targets [20]

These conditions necessitate the need to explore more sources for treating the disease.

Thus, many plants were known for their anticonvulsants activity, Various phytochemical and pharmacological studies have been carried out on these anticonvulsant plants .[18,19]

In earlier research, it was found that both *Withania somnifera* (L.)Dunal and *Bacoppa monnirei* have antiepileptic property, it can potentially be used for the treatment of epilepsy.

.

Withania somnifera (L.)Dunal *Withania somnifera*, known commonly as ashwagandha is an evergreen shrub in the Solanaceae or nightshade family that grows in India, the Middle East, and parts of Africa. This species is a short shrub growing 35–75 cm (14–30 inch) are small, green and bell-shaped. The ripe fruit is orange-red. The Latin species name *somnifera* means 'sleep-inducing'. The name *ashwagandha* is a combination of the Sanskrit words *ashva*, meaning 'horse', and *gandha*, meaning 'smell', reflecting that the root has a strong horse-like odor [17] . *Withania somnifera* has been used for more than 2500 years as a useful medicinal plant to improve disease defence, prevent aging, rejuvenate the body in a vulnerable situation, and generate a feeling of mental well-being [21].

Withania somnifera (L.) Dunal is an ancient Indian herbal drug which finds popular use as a convenient home remedies [22]. Roots of this plant have been extensively used in Ayurvedic and Unani systems of medicine over the years in geriatric tonics for its potent aphrodisiac, rejuvenate and life prolonging properties [23]. Anti-inflammatory, anticancer, antistress,

adaptogenic, endocrine and cardiovascular activities of *W. somnifera* root extract are reported.[24,25,26]. *W. somnifera* has also been shown to be effective in alleviating several central nervous system (CNS) disorders like epilepsy, anxiety, depression, catalepsy, morphine tolerance, tardive dyskinesia, and sleep [27,28,29]

The profound anticonvulsant activity of *W. somnifera* root extract as reported in various animal models is hypothesized to be through GABAA receptors. *W. somnifera* root extract is reported to have anxiolytic and CNS inhibitory effects. An experiment was conducted to determine the modulatory effect of a methanol extract of *W. somnifera* on the binding of [3H] GABA, [35S] tbutylbicyclophosphorothionate (TBPS)[30]. In the previous study it was suggested that the anticonvulsant effect of *Withania somnifera* against PTZ seizure threshold paradigm involved the $GABA_A$ergic modulation.[31]

In animal models for screening for anticonvulsant activity, it has been scientifically established that medicinal herbs used in traditional medicine for the treatment of epilepsy possess promising anticonvulsant properties and can be a source of newer anticonvulsants**.** **[32,33]**

In the previous study it was found that *W. somnifera* (Ashwagandha) comprise a broad spectrum of phytochemicals with a wide range of pharmacological properties.[34,35,36] *W. somnifera* seeds have reduced viability and germination rates, thus its regular cultivation method fails to achieve commercial demands mainly the production of desired phytoconstituents. Cultivation of plant cells/tissues under *in vitro* conditions and development of various biotechnological strategies will help to build an attractive alternative to provide adequate quality and quantity raw materials.

Currently, herbal medicines are gaining more attention due to their potential to treat a wide range of diseases. However, several challenges such as poor solubility, low bioavailability, inadequate oral absorption, instability, and unpredictable toxicity limit their effectiveness. To address these issues, "Nanotechnology" has introduced innovative therapies in the pharmaceutical field that can resolve the problems associated with herbal medicines. It is expected that combining natural products and herbal remedies with nanocarriers will significantly improve the effectiveness and importance of existing drug delivery systems.[37, 38, 39].

Medicinal plants and natural compounds, such as *Withania somnifera* (ashwagandha), *Ginseng*, curcumin, resveratrol, *Baccopa monnieri*, *Ginkgo biloba*, and Wolfberry have been applied to prevent or alleviate neurological diseases and relief of

neurological symptoms reported in *in vivo* or in clinical trials. Natural compounds in nanosize range as a therapeutic agent possess the same activity as in native state. Nanodrug delivery helps to increase the bioavailability of the drug and thereby specifically target cells and tissues. Nanoparticles, polymeric nanomicelles, complex polymers nanocrystal, and nanofibers are used to carry the medicinal plants for drug delivery system in the treatment of neurodegenerative diseases. This review is a study to understand the role of nanotechnology and natural compounds in associated with epilepsy. Neuroprotection refers to the strategies and relative mechanisms able to defend the central nervous system (CNS) against neuronal injury due to both acute (stroke and traumatic brain injury) and chronic neurodegenerative disorders (Alzheimer's disease and Parkinson's disease. With the ageing of the population, these pathologies and other brain disorders (Epilepsy and Dementia) are rising all around the world and thus represent a relevant health problem. In spite of many years of research and trials, there is no efficient cure for neurological diseases and current therapies available mainly provide symptomatic improvement. Many recent experimental observations lead to the conclusion that among all the mechanisms involved in neurological disorders, free radical damages may constitute a common important pathway leading to cell death. Antioxidant compounds are thus valuable candidates for the development of neuroprotective strategies. In fact, there is growing evidence that exposure to antioxidant compounds and relatives present in plants may provide health benefits.

Plant tissue culture is a pivotal technique in modern research, offering rapid cloning of plants, precise genetic modification, and the production of disease-free specimens. It plays a significant role in the conservation of endangered species, enhancement of crop yields, and the scalable production of valuable secondary metabolites, thereby advancing agricultural and biotechnological applications. Very limited literature of *Withania* tissue culture is available [40,41,42,43].

Bacopa monnieri (L.) commonly known as Brahmi, is a perennial herb that has been a cornerstone of Ayurvedic medicine for centuries. *Bacopa monnieri* is a small, creeping, perennial herb with succulent, prostrate stems and opposite, oblong leaves. The plant thrives in wet, marshy environments. It produces small, solitary, white or pale blue flowers with five petals. The fruit is an oblong capsule containing numerous tiny seeds. Its morphology supports rapid spread and adaptability in moist habitats. Traditionally, it has been used to enhance memory, reduce anxiety, and treat various neurological conditions. The therapeutic potential of *Bacopa monnieri* is attributed to its rich phytochemical composition, particularly

its bacosides, which are known to exhibit neuroprotective, antioxidant, and cognitive-enhancing properties.

Recent scientific research has begun to explore the potential anticonvulsant effects of *Bacopa monnieri*. Preclinical studies have shown promising results, suggesting that the herb may modulate neurotransmitter levels, enhance synaptic function, and provide neuroprotection against seizure-induced damage. These findings have sparked interest in the potential of *Bacopa monnieri* as a natural adjunct or alternative to conventional antiepileptic therapies.

This review aims to provide a comprehensive overview of the current research on the anticonvulsant properties of *Bacopa monnieri*. We will examine its phytochemical profile, and evaluate the evidence from preclinical and clinical studies.

Bacopa monnieri (L)Wettst, belonging to the Scrophulariaceae family and commonly known as Brahmi, is well known in India for its CNS activity but its neuropharmacological effect has not yet been explored.[44]. . The herb was believed to have been used by ancient Vedic scholars to memorize lengthy hymns and scriptures. In the past decades has been studied extensively [45,46,47] for its chemical constituents and its activity has been established in several *in vivo* and *in vitro* models; randomized clinical trials have also been carried out . Research on anxiety, epilepsy, bronchitis and asthma, irritable bowel syndrome and gastric ulcers also supports the Ayurvedic uses of *Bacopa* [48,49,50]. Moreover, plant tissue culture technique is an integral part of genetic transformation process, production of secondary metabolites, important bioactive compounds and production of somaclonal variants by callus culture Most significant application of callus tissue is accumulation of secondary metabolites which can be manipulated by biotransformation and number of other methods. Previous studies on plant tissue culture of *Bacopa monniera* have been carried out by many authors.[51,52,53,54].

Epilepsy is a chronic central nervous system disorder that occurs not only with the imbalance of glutamatergic neurons and inhibitory gamma-aminobutyric acid (γ-GABA) neurons, but also with abnormal Central cholinergic neuronal regulation. Previously it was reported that bioactive factors present in extract of *Bacopa monnieri* (L.)Wettst. offer neuroprotection by interrupting the pathological cascade that occurs during epileptogenesis.[45] There is some evidence to suggest that *Bacopa i*mproves memory free recall with evidence for enhancement in other cognitive abilities currently lacking perhaps due to inconsistent measures employed by studies across these cognitive domains.[46] This research has focused on its potential

anticonvulsant properties, which could offer an alternative or adjunct to conventional epilepsy treatments.

Furthermore, natural compounds in nano sizes range possess better neuroprotective activity. Consequently, polymeric nanomicelles, nanoparticles, and nanofibers of natural products are used in the treatment of neurodegenerative diseases.

The objective of this study will be to evaluate the preliminary phytochemical components, quantitative measures, and antioxidant activity of extracts from the *in vivo* roots, leaves, and *in vitro* cultivated tissues of *Withania somnifera* (L.)Dunal and *Bacopa monnirei*(L.)Wettst. During present investigation we also examine the anticonvulsant effect of nanoparticles made from *in vivo* root, leaf and *in vitro* grown tissues of *Withania somnifera* (L.)Dunal and *Bacopa monnirei*(L.)Wettst. Anticonvulsant activity will be evaluated against Maximum Electroshock (MES)-induced convulsions and pentylenetetrazole (PTZ)-induced convulsions in mice or rat models.

Problem to be investigated

Though *Withania somnifera* (L.)Dunal and *Bacopa monnirei*(L.)Wettst. is endowed with rich diversity of valuable biomolecules having wide range of medicinal activity, anticonvulsant activity of green synthesized nanoparticles (using extract of root, leaf and *in vitro* tissues) on animal model has not been reported so far. This research Investigate optimization of media and growth regulators for effective callus induction, shoot regeneration, and enhanced secondary metabolite production in *Withania somnifera* (L.)Dunal and *Bacopa monnirei*(L.).Wettst. This research also investigates several major obstacle related to the biochemical properties and anticonvulsant activity of green synthesized nanoparticles derived from *in vivo* root and leaf extracts and in-vitro grown tissues of *Withania somnifera* (L.)Dunal and *Bacopa monnirei*(L.)Wettst. The study aims to evaluate the biochemical differences between these sources, optimize the synthesis of green nanoparticles, and assess their anticonvulsant efficacy. A comparative analysis will also be conducted to determine whether nanoparticles from *in vivo* plantlets or *in vitro* grown tissues exhibit superior anticonvulsant properties in both plants.

Hypothesis

The hypothesis of this research is that green nanoparticles synthesized from in-vivo plantlets and in-vitro grown callus of *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst, exhibit significant anticonvulsant activity and possess unique biochemical properties that enhance their therapeutic potential. Optimizing growth media and regulators will enhance callus induction, shoot regeneration, and secondary metabolite production in both plants.

The rationale is that both *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst are well-known medicinal plants with established neuroprotective and anticonvulsant properties. By utilizing nanotechnology, specifically the synthesis of nanoparticles(synthesized by green methods), the bioavailability, stability, and overall efficacy of the plant-derived compounds can be improved.

It is anticipated that the biochemical investigation will reveal distinct profiles of phytoconstituents in the silver nanoparticles derived from both *in vivo* and *in vitro* sources, potentially indicating a difference in their therapeutic effects. The anticonvulsant activity of these nanoparticles will be evaluated using established models, such as the Maximum Electroshock (MES) and pentylenetetrazole (PTZ)-induced convulsion models in rats. It is expected that these nanoparticles will demonstrate superior anticonvulsant activity compared to traditional extracts, offering promising alternatives for epilepsy treatment. Ultimately, this study aims to provide a scientific basis for the development of novel, plant-based nanoformulations that offer safer and more effective alternatives for the treatment of epilepsy and related neurological disorders

Proposed investigation will help better to understand the detail *in vivo* plantlets and *in vitro* grown tissues along with their anticonvulsant activity of both plants. Study of secondary metabolites will help these plants to incorporate in new pharmaceuticals and in healthcare products.

Objective of Investigation

1. Identification and authentication of the both plants
2. Plant tissue culture of *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst. for procurement of cultured tissue.
3. Biochemical investigation of extracts of *in vivo* and *in vitro* grown plant parts of *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst.
4. Synthesis and Characterization of green synthesized nanoparticles of *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst. using different types of extracts.
5. Investigation of antioxidant activities of green synthesiszed nanoparticles of *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst.
6. Investigation of anticonvulsants effect of different extracts and green synthesized nanoparticles of *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst. on mice or rat model.

Research Design and Methodology

1. Tissue culture of *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst.

The research will optimize *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst. explant culture, testing different growth media, plant growth regulators(2,4-D, IAA,NAA, IBA, BAP, KN) and elicitors(salicyclic acid, benzothiadazole) to enhance callus induction, shoot regeneration, and secondary metabolite production in tissue cultures.

2. Preparation of extracts using different plant parts of *Withania somnifera* (L.)Dunal and *Bacopa monnirei*(L.)Wettst. from field grown plantlets and *in vitro* cultured tissues.

3. Phytochemical analysis of *in vivo* and *in vitro* grown plantlets extract for presence of secondary metabolites like Flavonoids, Saponins, Alkaloids, Phenols.

4. Green synthesis of nanoparticles using *in vivo* and *in vitro* grown plantlets extract of *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst.
5. Characterization of green synthesize nanoparticles - It can be done by various techniques such as UV-Vis Spectrophotometer, SEM, TEM, FTIR, XRD.
6. Antioxidant activity- DPPH and ABTS assays will be adopted for testing antioxidant activity

7. Toxicity test of green synthesized nanoparticles- Once toxicity levels of different extracts and its nanoparticles are established then safe doses are identified, proceed with anticonvulsant activity testing.

8. We need to obtain ethical approval for further research from IAEC(Institutional Animal Ethics Committee). We have to follow the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) or equivalent regulatory bodies.

9. Proceed to Anticonvulsant Testing - Anticonvulsant activity has to be done *in vivo* and *in vitro* grown plantlets derived green synthesized nanoparticles on Albino rat model. The anticonvulsant activity of these nanoparticles will be evaluated using established models, such as the Maximum Electroshock (MES) and Pentylenetetrazole (PTZ)-induced convulsion models in rats.

Tools to be used

- Plant/Animals models

For Anticonvulsant activity

Maximal Electroshock Seizure (MES) Test-

Mice/Rat

Pentylenetetrazol (PTZ) induce seizure

Mice/Rat

- Chemicals

MS Media, 2,4-D, KN, NAA, Benzene, Ethanol, Methanol, Toluene, Folin, Phenol reagent, Chloroform, SDA media (Pepton, Glucose and Agar) and Nutrient agar media, AgNPs, CuNPs, AuNPs, DPPH, ABTS

- Equipments

Curette Petridish, Weighing machine, microwave, culture tubes, water bath, filter paper, conical flask, beaker, glass rod, pH meter, centrifuge machine, autoclave, glass plate, measuring cylinder, test tube stand, micropipette, funnel, spatula, grinder, rotatory shaker, incubator, laminar air flow, UV-Vis spectrometer, Zeta potential, FTIR, XRD.

Contribution to the Existing Knowledge

This research significantly contributes to existing knowledge by exploring the enhanced unique biochemical properties and anticonvulsant activity of green nanoparticles synthesized from *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wetts.`It highlights how nanotechnology can improve the bioavailability, stability, and efficacy of plant-based treatments for epilepsy. By investigating both *in vivo* plantlets and *in vitro* grown tissues, the study provides a comparative analysis that can guide optimal nanoparticle synthesis methods. This work not only supports the development of more effective and safer epilepsy treatments but also bridges traditional herbal medicine with modern nanotechnology, fostering innovation in therapeutic approaches. This research also contributes to existing knowledge by optimizing *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst. Tissue culture techniques, enhancing callus induction, shoot regeneration, and secondary metabolite production, thus supporting large-scale propagation and therapeutic applications.

Significance of the work

The significance lies in advancing *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst .cultivation through efficient callus induction and shoot regeneration, leading to increased production of valuable secondary metabolites for medicinal use and sustainable large-scale propagation. This research explores the enhanced biochemical properties and anticonvulsant activity of green nanoparticles derived from *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst. By leveraging nanotechnology, it aims to improve the bioavailability, stability, and therapeutic efficacy of plant-based treatments for epilepsy. The study's comparative analysis of nanoparticles from *in vivo* plantlets and *in vitro* tissues provides insights into optimizing nanoparticle synthesis and application. This work could lead to safer, more effective treatments for epilepsy and potentially other neurological disorders, bridging traditional herbal medicine with modern nanotechnology.

Relevence to Society

This research holds significant relevance to society by addressing the urgent need for safer and more effective treatments for epilepsy, a debilitating neurological disorder affecting millions worldwide. By exploring the anticonvulsant potential of green nanoparticles synthesized from *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst. This study aims to develop plant-based nanoformulations with enhanced therapeutic properties and reduced side effects compared to conventional drugs. This research is relevant to society as it enhances the production of medicinal compounds from *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst. supporting the development of natural therapies, promoting health, and ensuring sustainable agricultural practices. The success of this research could lead to affordable, accessible, and natural alternatives for epilepsy treatment, improving patient outcomes and quality of life. Additionally, this work promotes the integration of traditional medicinal plants with cutting-edge nanotechnology, fostering innovation in healthcare and supporting sustainable medical practices.

Future Perspective

Future research could focus on optimizing metabolic yields and exploring genetic modifications to further enhance therapeutic potential and sustainability of *Withania somnifera* (L.)Dunal and *Bacopa monnieri* (L)Wettst. This research opens avenues for developing advanced plant-based therapies using green nanoparticles. Future studies could explore optimizing nanoparticle synthesis for other medicinal plants, enhancing their therapeutic efficacy. Additionally, this research could focus on large-scale production of nanoparticles, applications and clinical trials to establish safe and effective natural treatments for epilepsy and other neurological disorders.

References

1. Pramono E. The Commercialn use of Traditional knowlwdge and Medicinal plants in Indonesia.
2. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Edn 2. Spectrum Books Liomited,
3. Manchishi SM. Recent Advances in Antiepileptic Herbal Medicine. *Current Neuropharmacology*. 2017;16:79-83.
4. Ogurin OA. Epilepsy in Nigeria- A Review of Etiology, Epidemiology and Management. Benin Journal of Pastgraduate Medicine 2006;8(1): 27-51
5. E. Beghi The epidemiology of epilepsy, *Neuroepidemiology*, 54 (2020), pp. 185-191.
6. WHO.Epilepsy. https://www.who.int/news-room/fact-sheets/details/epilepsy.
7. Roger J P, Brian S M. Antiepileptic drug. In: Katzung BG, editor. *Basic and Clinical Pharmacology.* Fifth Edition. London: Prentice Hall International (UK) ltd; 1996. pp. 331–342.
8. Mattson R H. Drug treatment of partial epilepsy. *Adv Neurol.* 1992;57:643-650.
9. Samren E B, van Duijn CM, Koch S, et al. Maternal use of antiepileptic drugs and the risk of major congenital malformations: a joint European prospective study of human teratogenesis associated with maternal epilepsy. *Epilepsia*. 1997;38(9):981-990. doi:10.1111/j.1528- 1157.1997.tb01480.x.
10. Manyam, B. V. Epilepsy in ancient India. *Epilepsia* 33, 473–475 (1992).
11. The ayurvedic pharmacopoeia of India, Part II, Vol. II. Ministry of health and family welfare, Government of India, New Delhi, ISBN: 8190595202 (2008).
12. The ayurvedic formulary of India, Part I. Second revised ed., Ministry of health and family welfare, Government of India, New Delhi, ISBN: 8190115146 (2003).
13. Kumar S, Saxena G, Sudha A , Significance breakthrough in the field of anticonvulsant natural remedies, *Journal of the Maharaja Sayajirao of Baroda*, ISSN: 0025-0422, Vol-55, No.1(1) 2021.
14. Faaheem M, Ameer S, Khan A W, Haseeb M, Raza Q, Shah F A, Batiha G El-S, Koirala N, Adnan M, Assaggaf H, Alsiwiehri N O, A comprehensive review on antiepileptic properties of medicinal plants. *Arabian Journal of Chemistry*,Vol 15, issue 1, Jan 2022, 103478.
15. Mballaw A E, Kahawa I, Abdelgadir A A., Traditional approaches of medicinal plants in treatment of Epilepsy in West and East Africa, *Neuro Pharmac Journal* 5(2020) 121-130.

16. Junior L J Q, Almeida J R G S, Lima J T, Nunes X P, Siqueira J S, Olivera L E G, Almeida R N, Athayde-Filho P F, Barbosa-Filho J M, Plants with anticonvulsant properties – a review, *Brazilian Journal of Pharmacognosy*, 18(Supl): 789-819, Dec 2008.
17. Samren E B, van Duijn C M, Koch S, et al. Maternal use of antiepileptic drugs and the risk of major congenital malformations: a joint European prospective study of human teratogenesis associated with maternal epilepsy. *Epilepsia.* 1997;38(9):981-990. doi:10.1111/j.1528- 1157.1997.tb01480.x.
18. Chauhan AK, Dobhal MP, Joshi B 1988. A review of medicinal plants showing anticonvulsant activity. *J Ethnopharmacol 22*: 11-23.
19. Nsour W N, Lau CBS, Wong ICK 2000. Review on phytotherapy in epilepsy. *Seizure 9*: 96-107.
20. Manchishi SM. Recent Advances in Antiepileptic Herbal Medicine. *Current Neuropharmacology*. 2017;16:79-83.
21. Syed, Anees A., Reza, Mohammad I.; Singh, Pragati; Thombre, Ganesh K.; Gayen, Jiaur R. **Source:** Current Drug Metabolism, Volume 22, Number 7, 2021, pp. 561-571(11)
22. Sharma S. Dahunkar S and Karandikar S M, Effects of long term administration of the roots of ashwagandha (*Withania somnifera*) and shatavari (*Asparagus racemosus*) in rats, *Indian Drug*, 23 (1985)133.
23. Vaidyaratnam P S Varier's, Indian medicinal plants, A compendium of 500 species, (edited by PK Warrier, VPK Nambiar, Ramakutty) part II (Orient Longman Publications, Hyderabad) 1994, 52.
24. Rai D, Bhatia G, Sen T and Palit G, Anti-stress effects of Ginko and Panax gingseng a comparative study, *J Pharmacol Sci*, 93 (2003) 458.
25. Winters M, Ancient medicine, modern use*: Withania somnifera* and its potential role in integrative oncology, *Altern Med Rev*, 11 (2006) 269.
26. Rasool M and Varalakshmi P, Immunomodulatory role of *Withania somnifera* root powder on experimental induced inflammation: An *in vivo* and *in vitro* study, *Vascul Pharmacol*, 44 (2006) 406.
27. Naidu P S, Singh A and Kulkarni S K, Effect of *Withania somnifera* root extract on reserpine-induced orofacial dyskinesia and cognative dysfunction, *Phytother Res,* 20(2006) 140.

28. Bhattacharya A, Ghosal S and Bhattacharya S K., Anxiolytic antidepressant activity of *Withania somnifera* glycowithanoids: An Experimental study, *Phytomedicine*, 7 (2000) 544.
29. Dhuley J N, Nootropic-like effect of Ashwagandha (*Withania somnifera* L.) in mice, *Phytother Res*, 15(2001) 524.
30. Candelario M, Reyes-Ruiz J M, Darabedian N, Feimeng Z, Miledi R, Neustadt A R, Limon A, Direct evidence for GABAergic activity of *Withania somnifera* on mammalian ionotropic GABA A and GABA p receptors, *Journal of Ethanopharmacology,* Vol 171,2 Aug,2015, 264-272.
31. Kulkarni S K, Akula K K and Dhir A, Effect of Dunal root extract against pentylenetetrazol seizure threshold in mice:Possible involvement of GABAergic system, *Indian Journal of Experimental Biology*, Vol. 46, June 2008, pp.465-469.
32. Salama A, Elhadidy E, Abdalla A, Farag A R H, Effects of the aqueous seed extract of Withania somnifera (Ashwagandha) against pilocarpine-induced convulsions in rats. *Int. J. Pharm. Sci. Rev. Res*., Nov-Dec 2016, Article No 23, 116-121.
33. Raju S K, Basavanna P L, Nagesh H N, Shahbhag A D, A study on the anticonvulsant activity of *Withania somnifera* (Dunal) in albino rats, *National Journal of Physiology, Pharmacy and Pharmacology,* 2017, Vol 7, issue 1.
34. Uddin Q, Simiulla L, SinghV K, Jamil, S S, Phytochemical and Pharmacological Profile of *Withania somnifera* Dunal : A Review, *Journal of Applied Pharmaceautical Science,* 02(01); 2012: 170-175.
35. Saleem S , Muhhamad A, Hussain M A, Altaf M and Bukhari S N A, *Withania somnifera* L:Insights into the phytochemical profile, therapeutic potential, clinical trial and future prospective, *Iran J Basic Med Sci* 2020 Dec. 23(12): 1501-1526.
36. Lerose V, Ponticelli M, Beneditto N, Carlucci V, Lela L, Tzvetkov N T and Milella L, *Withania somnifera* (L.) Dunal, a potential source of Phytochemicals for treating Neurogenerative Diseases: A Systematic Review, *Plants* 2024, 13(6), 771.
37. Harika P, Deepthi B V P, Baherji R, Ali J, Sharma J V C, Herbal Nanoparticles, *World Journal Pharmaceutical and Medical Research,*2021, 7(3), 127-130.
38. Manohar P S, Khedkar R E, Adhav A, The green synthesis of silver nano-particle by plant extract, *Int. J of Pharm. Res. And Appli*., Vol 8, issue 1, 2023, 552-562.
39. Dhabian S J, Jasim R S, Anticancer and Antioxidant Activity of the Greenly Synthesized Zinc Nanoparticles Composites using Aqueous Extracts of *Withania somnifera* plant, Egyptian Journal of Chemistry, Vol. 64, No 10, 5561-5574(2021).

40. Adhikari S R, Pant B, Induction and Proliferation of in vitro mass of callus of *Withania somnifera* (L.) Dunal, *Research in Plant Sciences,*2013, Vol 1, No.3, 58-61.
41. Saema S, Ahmad I Z, Misra P, Rapid in vitro plant regeneration from nodal explants of Withania somnifera(L.) Dunal: a valuable medicinal plant, International Journal of Science and Research,Vol-4 Issue 6, June 2015.
42. Gaurav N, Singh A P, Srivastava A, Kumar A Gariya H S, In vitro propagation of *Withania somnifera L*. (Dunal) fromc callus of embryonic cotyledon explants in B5 medium, Indian Forester, 144(1): 36-40, 2018.
43. Ramesh S, Karthikeyan K, Muruganantham P, Chandran C, In vitro regeneration of *Withania somnifera* L. dunal. A rare medicinal plant, Current Research in Interdisciplinary Studies, Vol 1, Issue 3, 1-8,2022.
44. Kausik D, Tripathy A, Tripathy R, Ganachari M, Khan S A, Anticonvulsant activity of *Bacopa monnieri* in rodents, Brazillian Journal of PharmaceuticalSciences, Vol 45.n.4.oct/dec, 2009.
45. Mohapatra H P, Rath S P (2005). In vitro studies of Bacopa monnieri-An important medicinal plant with reference to its biochemical variation. *Indian J. Expt Bio.* Vol.43 :373-376.
46. Rajani M, Shrivastava N , Ravishankara M N (2004). Brahmi [*Bacopa monnieri* (L.) Pennell .] - a MedhyaRasayana drug . In: New Concepts in Medicinal Plants . Science Publishers, Enfield (NH) , USA, PP- 89- 110.
47. Ganzerra M, Gampenrieder J, Pawar R, Khan I, Stuppner H (2004). Separation of major triterpenoidsaponins in *Bacopa monnieri* by high performance liquid chromatography. *Anal Chim Acta* 516: 149-154.
48. Roodenrys S, Booth D, Bulzomi S, Phipps A (2002). Chronic effects of Brahmi *(Bacopa monnieri*) on human memory . *Neuropsycopharmacology* 27 (2): 279-281.
49. Srinivasa H, Bagul M., Padh H , Rajani M (2004). A rapid densitometric method for the quantification of luteolin in medicinal plants using HPTLC . *Chromatographia* 60 (1/2) : 131-134.
50. Prakash Om, Singh G, Singh R, Mathur S, Bajpai M, Yadav S (2008). Determination of Bacoside A by HPTLC in Bacopa monnieri extract . Int. Journal of Green Pharmacy 2 (3) : 173-175.
51. Nisha K K, Seetha K, Rajmohan K., Purushottam M G (2003). Agrobacterium tumefaciens mediated transformation of Brahmi, *Bacopa monnieri (L.)* Wettst., A popular medicinal herb of India *Curr. Sci.* 85 (1):85-88.

52. Mehta A., Phytochemical screening of *in vivo* and *in vitro* grown plantlets of *Bacopa monnieri* L.International Journal of Applied and Natural Sciences, Vol 6, Issue 4, July 2017:129-138.
53. Mehta A., Growth and development of rooted plantlets of *Bacopa monnieri* L. in auxin free tissue culture medium, *International Journal of Botany and Research*, Vol 7, Issue-3, June 2017, 11-16.
54. Mehta A., Effect of plant growth regulators on callus multiplication and in vitro plant regeneration in *Bacopa monnieri* L., *International Journal of Medicinal Plant Research*, Vol 6(5),May 2017, 337-345
55. Kamali K, Venkataramaiah Ch, Rajendra W, Antiepileptic potential of *Bacopa monnieri* in the rat brain during PTZ-induced epilepsy with reference to cholinergic system and ATPases, Journal of Traditional and Complementary Medicine, Vol 11, Issue 2,March 2021, 137-143.
56. The cognitive enhancing effects of *Bacopa monnieri*-A systematic Review of Randomized, Controlled Human Clinical trials, The Journal of Alternative and Complementary Medicine, Vol 18, No. 7, 2012, 1-8.